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Variability in the Fetal Hemoglobin Level of the Normal Adult

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We analyzed blood samples from more than 200 normal adults, and quantified their Hb F by cation-exchange high-performance liquid chromatography. In several subjects with slightly elevated Hb F (0.4–4.3%), we determined the $^{G}\gamma$ levels in the Hb F and DNA sequence variations in the locus control region II and in the $^{G}\gamma$ and $^{A}\gamma$ promoters. About 25% of the ~200 normal teenaged high school students had elevated Hb F; detailed analyses of some 20 students, selected at random, identified most as females with a homozygosity for the C→T variation at position −158 ($^{G}\gamma$). One 11-year-old boy was heterozygous for the A→G change at position −161 ($^{G}\gamma$); he and two of his relatives had ~4% Hb F, high $^{G}\gamma$ values, and a high level of (mainly) $^{G}\gamma$-mRNA. Nearly 40 normal adults from Macedonia and from Georgia (mostly Caucasians) were tentatively identified as Swiss HPFH heterozygotes because slightly elevated Hb F levels were observed at least once. Many of these persons were heterozygous or homozygous for the C→T mutation at −158 ($^{G}\gamma$), and a few carried a γ-globin gene triplication. The C→T change appears to be an important factor predisposing the adult to increased Hb F production. Evidence suggests a gene dose effect in (mildly) anemic adults; however, other factors besides the C→T change at −158 ($^{G}\gamma$), including factors not linked to the β-globin region, may cause an increase in γ-chain synthesis. © 1996 Wiley-Liss, Inc.

Key words: Hb F, $^{G}\gamma$ chain, gene activation, $^{G}\gamma$ promoter mutations, γ gene triplications, mild anemia

INTRODUCTION

There are quite a number of conditions, besides hereditary persistence of fetal hemoglobin (HPFH), β-thalassemia (thal), δβ-thal, and sickle-cell anemia, in which slight elevations of fetal hemoglobin (Hb F) have been observed [1]. They are often referred to as Swiss-type HPFH [2, and references quoted therein] or heterocellular HPFH. There is little doubt that this condition consists of several anomalies such as γ-globin gene rearrangements and γ-globin gene triplications [3], certain mutations in the $^{G}\gamma$ promoter [4], and the presence of a C→T substitution at position −158 ($^{G}\gamma$) or of a G→A change at position −161 ($^{G}\gamma$) [5,6], all being linked to the β-globin gene complex. In addition, nonlinked factors may exert an influence [1].

Here we provide data about the Hb F levels, $^{G}\gamma$ levels, and DNA sequence variations for a relatively large number of normal healthy adults who had their Hb F levels determined with one of the more advanced high-performance liquid chromatographic (HPLC) procedures. A surprisingly large number of subjects had increases in Hb F, the molecular basis of which was diverse and complex.

MATERIALS AND METHODS

Blood samples were collected in vacutainers with EDTA as anticoagulant and transported in ice to the laboratory, or shipped by fast air-mail service from Skopje, Republic of Macedonia to Augusta, GA. Informed con-

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sent was obtained. Hematological data were obtained with an automated cell counter.

Each sample was studied by isoelectrofocusing (IEF) [7]. Quantitation of Hbs A_2 and F was by cation-exchange HPLC [8,9]. The column used was either a polyCAT-A (200 × 4.6 mm I.D.; 5 μm particle size; PolyLC, Columbia, MD) or a SynChropak CM-300 (250 × 4.6 mm; SynChrom, Inc., Linden, IN) column; the elution system has been detailed previously [9,10]. Hb F elutes ahead and completely separates from Hb A_{1c} in the SynChropak system, while this sequence is reversed on a poly-CAT-A column. Both methods are reproducible at the 0.1% Hb F level, and as little as 0.1% Hb F can be detected. The method is sensitive to (small) changes in the composition of the developers and, to a lesser extent, to variation in temperature.

The small amount of Hb F increased to 0.4–4.0% in several individuals, was isolated from the appropriate red-cell lysates by DEAE-cellulose chromatography [11] or by polyCAT HPLC [10], and its γ-chain composition was determined by reversed-phase HPLC [12,13]. Accurate data could be obtained in about 90% of all cases.

DNA was isolated from selected samples by the method of Poncz et al. [14]. The presence of possible mutations in the 5′ HS-2 (positions −10924 (G or T) and −10905 (A or G)), the $^{G}\gamma$ promoter (positions −369 (C or G), −309 (A or G), −161 (A or G), and −158 (C or T)), and the $^{A}\gamma$ promoter (positions −657 (G or T) and −271 (C or T)) was determined by dot-blot analysis with ^{32}P-labeled oligonucleotide probes specific for the listed mutations [15–17] or by sequencing of amplified segments of DNA as described elsewhere [15,16]. The resulting "haplotypes" are listed as #3, #3A, #19, and #19A [17]. Nucleotides (nts) at these positions that are specific for each haplotype are:

	← HS-2 →		← $^{G}\gamma$ →				← $^{A}\gamma$ →	
	−10924;	−10905	−369;	−309;	−161;	−158	−657;	−271*
Type #3	T	A	C	A	G	T	G	C
Type #3A	T	A	C	A	G	C	G	C
Type #19	G	G	G	G	G	C	T	C
Type #19A	T	A	C	G	G	C	T	C

Blood samples from 2 teenagers and some of their relatives were (re)collected and (re)evaluated, including the determination of relative quantities of γ- and β-mRNAs with the reverse transcription polymerase chain reaction (RT-PCR) procedure described elsewhere [18].

RESULTS

The studies reported here include data from two collections. The first concerns Caucasian and Black high school students who participated in a testing program for Hb abnormalities. Those who were found to be normal by IEF and had normal hematological values were selected for this Hb F screening program. The second group of so-called Swiss HPFH heterozygotes consisted of healthy adults without a hemoglobinopathy who were thought to have slightly elevated levels of Hb F either by starch gel electrophoresis or by IEF; data for 12 subjects have been reported before [2].

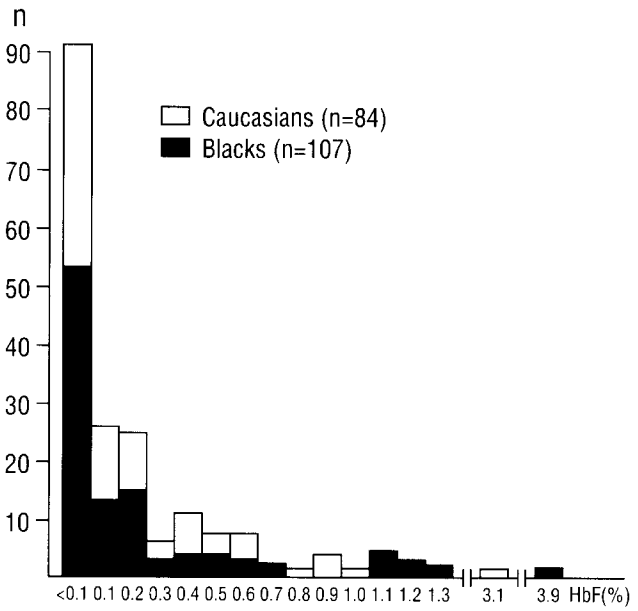

Fig. 1. Relationship between levels of Hb F (in percentage and determined by cation-exchange HPLC) and numbers of healthy high school students participating in the survey.

Survey of High School Students

Blood samples from 191 subjects (98 females, 93 males) are included; 84 were Caucasian and 107 were Black. None had an abnormal Hb or a β-thal (Hb A_2 below 3.5%; no microcytosis or hypochromia), but the possible presence of an α-thal heterozygosity (mainly α-thal-2 trait or −α/αα) was not excluded. Figure 1 summarizes the Hb F data; nearly 50% of the students (91 out of 191) had no detectable Hb F, while some 26% (50 out of 191) had borderline levels of 0.1% and 0.2%. As many as 50 students (25 Caucasians and 25 Blacks) had Hb F levels of 0.3% and higher, with 2 males having 3.1% and 3.9% Hb F, respectively. Table I summarizes data for 20 students (11 Caucasians, 9 Blacks) with the highest Hb F levels (0.5–3.9%); selection of these individuals was based solely on the availability of a second blood sample. Their hematological data fell within normal range, although a mild microcytosis and hypochromia could be observed in some, perhaps of a (mild) Fe deficiency anemia (about 30% of high school students in Georgia have such a deficiency [19], but none of the students participating in this survey received supplementary iron). The $^{G}\gamma$

TABLE I. High School Students With Normal Hematology and Slightly Elevated Levels of Fetal Hb

Subject	Sex-age	Race	Hb (g/dl)	RBC (10^{12}/l)	MCV (fl)	MCH (pg)	Hb A_2 (%)	Hb F (%)	$^{G}\gamma$ (%)	−158 $^{G}\gamma$[a]
S-2133	M-17	C	15.9	5.08	90.2	31.3	2.4	3.1	79.2	TT
S-2112	F-17	C	13.7	4.85	86.4	28.2	1.2	1.0		
S-2111	F-13	C	13.3	4.16	94.0	32.0	3.4	0.9	70.5	TT
S-2023	F-18	C	12.1	3.85	90.9	31.4	1.6	0.9		
S-1867	F-15	C	14.2	4.63	92.0	30.7	3.1	0.9	64.2	TT
S-2022	F-18	C	13.4	4.31	90.3	31.1	2.7	0.8		
S-1851	M-18	C	15.4	4.93	90.1	31.2	3.0	0.6	57.8	TT
S-2063	F-14	C	12.2	3.99	91.0	30.6	2.9	0.6	65.6	TT
S-1968	F-16	C	13.3	4.24	89.4	31.4	2.0	0.6		
S-1986	F-15	C	14.2	4.93	85.8	28.8	2.2	0.5	69.8	TT
S-1865	F-15	C	14.0	4.76	87.0	29.4	2.7	0.5	61.6	TT
S-1823	F-16	B	11.8	4.50	81.8	26.2	1.6	1.3	47.2	
S-2089	F-14	B	12.7	4.83	78.1	26.3	2.9	1.2	55.0	TT
S-1852	F-16	B	11.3	4.27	79.4	26.5	1.8	1.1	67.7	TT
S-2068	F-14	B	13.9	4.27	97.7	32.6	2.3	1.1	58.3	
S-2059	F-14	B	11.2	4.09	80.1	27.4	1.6	0.7		CT
S-2100	M-14	B	15.6	5.28	86.2	29.5	3.0	0.6	29.1	CC
S-2073	F-15	B	12.9	4.49	88.4	28.7	3.3	0.5	30.0	CC
S-2010	F-17	B	12.8	4.41	87.3	29.0	1.8	0.5	48.2	CT
S-128	M-11	B	13.2	4.36	92.2	30.3	2.9	3.9	67.5	CC[b]

[a]Additional evaluated variations in the $^{G}\gamma$ promoter were at −309 (A→G); −401 (7 bp deletion); −1105 (C→T); and −1106 (G→T) [15]. All had the expected sequences (AA at −309; no deletion at −401; CC at −1105; GG at −1106), except S-2059 (heterozygous for the 7-bp deletion at −401; CT at −1105; GT at −1106), S-2073 (AG at −309; homozygous for the 7-bp deletion at −401; TT at −1105; TT at −1106), and S-128 (AG at −309).
[b]See Table II.

values are included for 15 students, and the nts at position −158 of the $^{G}\gamma$-globin genes (C or T) are listed for 14 students. All but a few were homozygous for T at −158 ($^{G}\gamma$), 2 were heterozygous for the C→T change, while three were homozygous for C at −158 ($^{G}\gamma$). High $^{G}\gamma$ values correlated with T at −158 ($^{G}\gamma$) except for student S-128, who had C at −158 ($^{G}\gamma$) on both chromosomes but a high $^{G}\gamma$ value of 67.5%. As many as 16 students (80%) were females.

Data for a few family members of the 2 students with higher Hb F levels are listed in Table II; included are the nts observed at positions −158 and −161 of the $^{G}\gamma$ promoter (determined by sequencing of amplified DNA), and the relative levels of γ- and β-mRNAs, and of $^{G}\gamma$ and $^{A}\gamma$-mRNAs. All 3 members of S-2133's family had TT at −158 ($^{G}\gamma$) and GG at −161 ($^{G}\gamma$), with variable levels of Hb F (0.3–3.1%), high $^{G}\gamma$ values, variable γ-mRNA levels (high in student S-2133 but not in his brother and sister), and high $^{G}\gamma$ to $^{A}\gamma$-mRNA ratios. In contrast, the 3 members of S-128's family had Hb F levels of 3.9–4.2%, with high $^{G}\gamma$ values (67–79%), and increased γ-mRNA levels (8.4–13.4%, with 3.2% for a control), with high $^{G}\gamma$-mRNA values (68–77%), while homozygosity was observed for C at position −158 ($^{G}\gamma$) and heterozygosity for G at −161 ($^{G}\gamma$) (normal is A at this position of the $^{G}\gamma$ promoter).

Swiss HPFH Heterozygotes

The total number included in this study was 39; only 6 were black and 33 were Caucasian, and all were age 10 years or older. Selection was by an inspection of a starch-gel electrophoretic separation (by G.D.E. in the Republic of Macedonia) or by IEF (by J.Ye.L. in Augusta, GA); only the samples with a detectable elevation of Hb F were included. Each sample was analyzed by cation-exchange HPLC, and the $^{G}\gamma$ and $^{A}\gamma$ percentages in the Hb F were determined by reversed-phase HPLC as described before. Furthermore, the nts at position −158 ($^{G}\gamma$) were determined for all 39 samples, either by dot-blot analysis hybridizing amplified DNA with ^{32}P-labeled oligonucleotide probes, or by sequencing of amplified DNA. Haplotyping was limited to two positions in the locus control region (LCR) HS-2, three positions in the $^{G}\gamma$ promoter, and two positions in the $^{A}\gamma$ promoter (see Materials and Methods). All chromosomes had nts at these positions specific for haplotype #3 [17], with the exception of that at −158 ($^{G}\gamma$); a C at this position was characteristic for haplotype #3A, and a T for haplotype #3. Figure 2 summarizes the data; of the 39 cases studied, 12 had haplotypes 3A/3A (CC at −158, $^{G}\gamma$), 14 had haplotypes 3/3A (CT at −158, $^{G}\gamma$), and 13 had haplotypes 3/3 (TT at −158, $^{G}\gamma$). Subjects S-128 with haplotypes 3A/3A (CC at −158, $^{G}\gamma$) had a high $^{G}\gamma$ value and high Hb F value, while the average Hb F and $^{G}\gamma$ values for the other 11 cases were 1.6% and 40.1%, respectively. Two of the 14 subjects with haplotypes 3/3A (CT at −158, $^{G}\gamma$) had about 4% Hb F and high $^{G}\gamma$ values (>90%); both were found to carry a γ-globin gene triplication on one chromosome ($+^{G}\gamma\cdot-^{G}\gamma\cdot^{A}\gamma$, or type III in [20]). The average Hb F and $^{G}\gamma$ values for the remaining 12 subjects were 1.5%

TABLE II. Hematological Data for a Few Family Members of Two High School Students With Elevated Fetal Hb

Subject	Sex-Age	Relationship	Hb (g/dl)	RBC (10^{12}/l)	MCV (fl)	MCH (pg)	Hb A_2 (%)	Hb F (%)	$^{G}\gamma$ (%)	−158 $^{G}\gamma$	−161 $^{G}\gamma$	←mRNA (%)→[a] $\gamma/(\gamma+\beta)$	$^{G}\gamma/(^{G}\gamma+{}^{A}\gamma)$
S-2133	M-17		15.9	5.08	90.2	31.3	2.4	3.1	79.2	TT	GG	13.3	71.2
A.C.	M-22	Brother	15.2	5.02	87.5	30.3	2.4	0.3		TT	GG	2.6	63.4
B.C.	F-14	Sister	13.5	4.21	87.6	32.1	2.7	1.8	57.5	TT	GG	0.9	62.2
S-128	M-11		13.2	4.36	92.2	30.3	2.9	3.9	67.5	CC	AG	10.5	67.9
M.H.	F-5	Sister	12.8	4.19	88.5	30.5	2.8	4.2	75.8	CC	AG	8.4	75.7
T.H.	M-31	Father	15.6	5.21	92.5	29.9	2.6	3.9	78.6	CC	AG	13.4	77.4

[a]One sample from a normal adult female (Hb F = 0.1%) gave these values: $\gamma/(\gamma+\beta)$ = 3.2%; $^{G}\gamma/(^{G}\gamma+{}^{A}\gamma)$ = 17.0%.

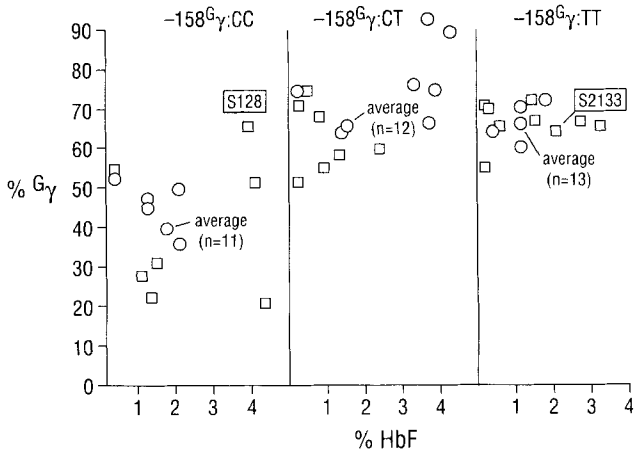

Fig. 2. Relationship between levels of Hb F (in percentage and determined by cation-exchange HPLC) and $^{G}\gamma$ values in three groups of healthy adults characterized by the nt (C or T) at position −158 of the $^{G}\gamma$ promoter. Blocked-in values are not included in the calculation of the average values but are discussed in the text. S-128 and S-2133 refer to 2 young adults listed in Tables I and II. Circles, females; squares, males.

and 66.3%, respectively. Finally, the average Hb F and $^{G}\gamma$ values for the 11 subjects with haplotypes 3/3 (TT at −158, $^{G}\gamma$), which included subject S-2133 listed in Tables I and II, were 1.2% and 66.0%, respectively. As many as 13 of the 39 participants (33%) had Hb F levels below 1%, indicating the relative insensitivity of the electrophoretic methods for selecting the Swiss type of HPFH carriers. No differences were observed between the Hb F and $^{G}\gamma$ values of males and females.

Several of the Swiss HPFH heterozygotes from Macedonia had been analyzed 8 years before, and Table III compares the data obtained at that time with the more recent information; both sets of data were obtained by the same HPLC methodology. In all instances the Hb F level had sometimes decreased to extremely low values; these differences might in part be due to the methodology that was used (alkali denaturation in 1986, and cation-exchange HPLC in 1994). No such change was observed for the $^{G}\gamma$ values, although some large differences were seen for some individuals (R-193, R-200, R-231, and R-741T) that may have been due to difficulties in obtaining an enriched Hb F sample from a red-cell lysate with extremely low levels of fetal Hb.

DISCUSSION

The survey of nearly 200 (Caucasian and Black) teenagers confirms that many normal, healthy adults may have slightly elevated levels of Hb F (0.3–1.3%); the incidence appears to be about 25%. Occasionally higher Hb F values (>3%) are recorded, but these are likely due

TABLE III. Fetal Hb and $^{G}\gamma$ Values for Macedonian Adults With Swiss-Type HPFH, Collected in 1986 [2] and 1994

Subject	Year	Hb F (%)	$^{G}\gamma$ (%)	−158 $^{G}\gamma$	Subject	Year	Hb F (%)	$^{G}\gamma$ (%)	−158 $^{G}\gamma$
R-182	1986	5.0	65.5		R-1007	1986	1.7	29.1	
	1994	3.4	67.2	TT		1994	1.0	28.3	CC
R-206	1986	2.4	59.6		R-231	1986	1.7	51.5	
	1994	0.2	50.8	CT		1994	<0.1	71.2	CT
R-193	1986	1.9	58.6		R-216	1986	1.5	48.9	
	1994	<0.1	71.0	TT		1994	<0.1		CC
R-212	1986	1.2	47.8		R-741[a]	1986	6.3	73.4	
	1994	0.2		CT		1994	1.8	72.3	TT
R-200	1986	1.6	53.4		R-741M[a]	1986	3.2	80.9	
	1994	0.7	68.9	CT		1994	<0.1	84.6	TT
R-123	1986	2.6	64.0		R-741T[a]	1986	1.0	56.0	
	1994	1.5	71.2	TT		1994	<0.1	71.9	TT

[a]Subjects with hereditary spherocytosis; patient R-741 was 2 years old in 1986. The father (R-741T) was considered normal.

to a mutation in either the $^{G}\gamma$ or $^{A}\gamma$ promoter sequences, resulting in the so-called nondeletional HPFH (reviewed in [1]). Methodology plays an important role in obtaining the data. The most commonly used alkali denaturation technique will record overly high numbers in adults with low levels of Hb F [9], while the cation-exchange HPLC procedure, developed by Bissé and Wieland [8] and used in this study, may well be the most sensitive and accurate method presently available.

No single reason can be given for the increase in Hb F production seen in the survey of the high school students or in the several individuals with the so-called Swiss-type HPFH (Fig. 2). A few may have one of the mutations in the γ promoter that are known to elevate γ-chain production, but none were observed in our survey. Others may have γ-globin gene rearrangements or γ-globin gene triplications [3]; 2 such individuals were detected. An interesting family is that of S-128 (Table II). This is the second observation of the A→G mutation at position −161 ($^{G}\gamma$), and the data for the 3 carriers are quite similar to those made earlier [6]. A small but distinct elevation of Hb F (about 4%) was present in all three heterozygotes with high $^{G}\gamma$ values (68–79%). The elevated level of γ-mRNA (8–13% of total γ + β-mRNA) indeed indicates an increased activity of γ-globin genes (primarily the $^{G}\gamma$-globin gene). The precise mechanism by which this mutation causes an increase in γ-mRNA and γ-chain production is not known, but a change in binding of a regulatory protein is a possibility [21].

Other factors that appear to play a role are the C→T mutation at position −158 ($^{G}\gamma$), the sex of the individual, the number of α-globin genes, the F-cell production locus at Xp22.2–22.3 [21–24], and the presence of a (mild and undefined) anemia. The effect of the C→T change is not consistent, and higher Hb F levels are not observed in all individuals with a homozygosity for T at −158 (Table II) [2]. Moreover, individuals with an increased Hb F level and homozygosity for T at −158 ($^{G}\gamma$) might have normal Hb F percentages when reexamined at a later time (Table III). However, it appears likely that the C→T changes at −158 ($^{G}\gamma$) is one of the factors predisposing the adult to higher levels of Hb F and an increase in γ-mRNA and γ-chain synthesis, particularly when the erythropoietic system is challenged as in severe anemia. An excellent example is given in Table IV. Both children with a Hb Mizuho heterozygosity [β68(E12)Leu→Pro] suffer from severe anemia because of the instability of this variant [25,26]. The 2 patients are both 5-year-old males, and their haplotypes are identical except for the nts at −158 ($^{G}\gamma$), which are TT (haplotypes 3/3) in subject D.C. with 21–22% Hb F and high $^{G}\gamma$ of 74%, and CC (haplotypes 3A/3A) in subject R.A. with 10% Hb F and low $^{G}\gamma$ of 30–31%. Indeed, one is tempted to assume that patient D.C. is a high Hb F responder because of the homozygosity for T at the −158 ($^{G}\gamma$) position. Another example is given in Figure 3, where Hb F and $^{G}\gamma$ values for 26 β-thal heterozygotes with the −29 (A→G) allele are presented. This β-thal mutation, first described in 1984 in American Blacks [27], was found on two haplotypes, namely type #3 (with T at −158, $^{G}\gamma$) and type #19A (with C at −158, $^{G}\gamma$); see Materials and Methods for details about these two haplotypes. Most heterozygotes have the mutation on haplotype #3 (T at −158, $^{G}\gamma$); their Hb F value varies between 0.7–8.9%, with high $^{G}\gamma$ values (48–76%) which are, to some extent, influenced by the haplotype of the normal chromosome. The four heterozygotes with the mutation on haplotype #19A (members of family D are excluded) have low levels of Hb F (0.7–1.9%), low $^{G}\gamma$ values (14–24%), and C at −158 of the $^{G}\gamma$ gene of the chromosome with the β-thal allele. This comparison supports the idea that the presence of T at −158 on the β-thal chromosomes promotes Hb F (mainly $^{G}\gamma$) production during a period of hematopoietic stress. The data for the 5 members of family D, each with the same β-thal allele on a chromosome with haplotype #19A (C at −158, $^{G}\gamma$) and a normal chromosome with haplotype #3 (T at −158, $^{G}\gamma$) lend support to this suggestion. A direct relationship between percentage of Hb F

TABLE IV. Two Children With Hb Mizuho (β68(E12)Leu→Pro) Heterozygosity and Different Levels of Fetal Hb*

Subject	Sex-Age	Origin	Hb (g/dl)	RBC (10^{12}/l)	PCV (l/l)	Hb A_2 (%)	Hb F (%)	$^{G}\gamma$ (%)	−158 $^{G}\gamma$
D.C.	M-5	Kentucky	7.2	3.05	0.257	2.8	21.6	74.1	TT
R.A.	M-5	Holland	4.9	1.76	0.199	3.0	9.8	30.5	CC

*Samples collected prior to blood transfusion; data are from Refs. 25 and 26.

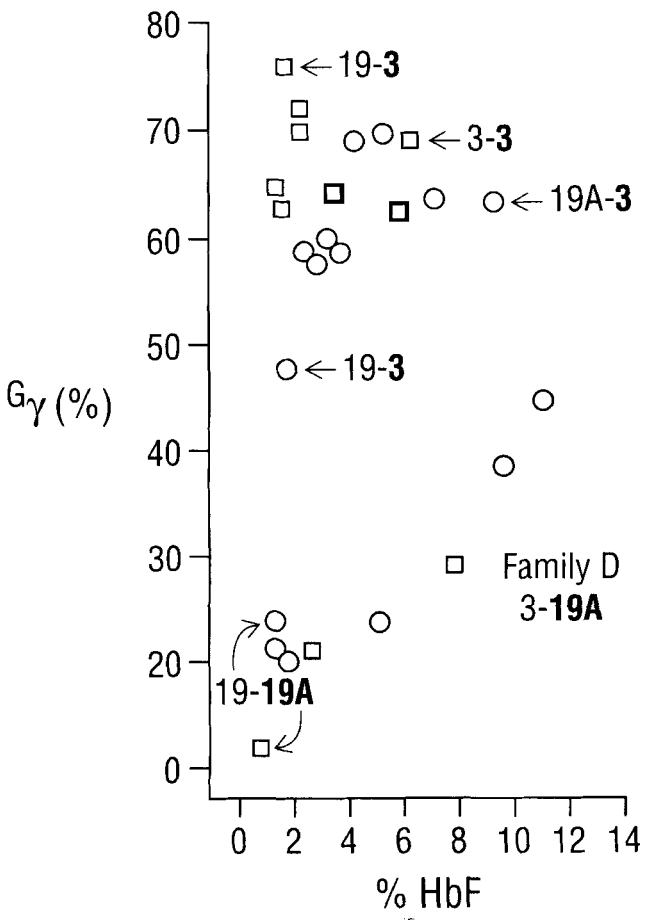

Fig. 3. Relationship between levels of Hb F (in percentage and determined by cation-exchange HPLC) and $^{G}\gamma$ values in 26 β^{+}-thal heterozygotes with the −29 (A→G) allele. Numbers 3, 19, and 19A refer to haplotypes; family D is discussed separately in the text. Haplotype indicated by bold numbers carries the β-thal allele.

and percentage of $^{G}\gamma$ can be noted, indicating activation of the $^{G}\gamma$-globin gene of the normal chromosome with haplotype #3 (T at −158, $^{G}\gamma$) in some of these individuals (the 2 females with the higher Hb F levels of 9.5% and 10.7% had the more significant anemia).

Sixteen of the 20 randomly-selected teenagers with normal hematology but elevated Hb F were female students, and most were homozygous for T at −158 ($^{G}\gamma$) (Table I). Menstrual blood loss and pregnancy (3 students were known to be pregnant; a pregnancy survey for the entire group of students could not be conducted) may cause a sufficient erythroid stress to promote a slight increase in γ-globin gene activation, mainly in females with the C→T change at position −158 of the $^{G}\gamma$ promoter, confirming data reported before [28].

In conclusion, the slight elevation of the level of Hb F in many normal adults is multifactorial. Important factors appear to be the −158 (C→T) and the −161 (G→A) mutations in the promoter of the $^{G}\gamma$-globin gene, which predisposes to increased γ-chain synthesis under mild erythroid stress. The temporary effect might be gene dose-related because most healthy adults with increased Hb F are homozygous and a few are heterozygous for one of these mutations, and the temporary effect is observed in both males and females. Our conclusions are in agreement with those of Month et al. [29], who also decided that the C→T base change at −158 ($^{G}\gamma$) might be important for gene expression, but that hemolytic stress is required for an effect on γ gene expression. A smaller survey of 4 normal adults with low Hb F levels (<0.2% by alkali denaturation) and 4 with slightly higher levels of Hb F (0.8–1.2% by alkali denaturation) failed to demonstrate the presence of $^{G}\gamma$ promoter mutations in the latter [30], while significant differences were seen in F-cell percentages. These observations support the concept that the slight increase in Hb F level in some normal adults is multifactorial.

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